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Patentanmeldung Nr. Patent application No. Demande de brevet n°

02017036.1

Der Präsident des Europäischen Patentamts;
Im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets
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The Provost Fellows and Scholars of the College of the Holy Undivided Trinity of Queen Elizabeth Nea
Dublin 2
IRELAND

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"Biochip Assembly"

Introduction

The present invention relates to a biochip assembly for use in biological assays.

Biochemical, microbiological, chemical and many other assays are performed every day in laboratories. While a considerable amount of attention has naturally been placed on such biological cell assaying for humans, this is also becoming more important in the field of animal welfare and indeed plant production generally.

A rapidly advancing research area in biology is the study of cell receptor-ligand interactions resulting in cell-substratum and cell-cell adhesion followed by subsequent cell migration. The pre-requisite to transendothelial migration of certain cell lines into sites of infection is paramount to the study of inflammatory diseases. This can be briefly summarised as cell flow and rolling, tethering and activation of integrin receptors which is a key recognition step, attachment to the endothelial ligands via activated integrins and finally transendothelial migration or diapedesis. Unfortunately, to date, most of the assay techniques are not particularly successful for the study of these mechanisms. Currently, the majority of studies involving cell rolling and chemokine induced cellular arrest have utilised capillary systems wherein cell flow and shear stress are controlled utilising syringe pumps. Such observations are constrained by a number of factors. Firstly, the relative large (> 100 μm) size of the standard glass capillaries limits the physiological analogies to the proximal microvascular regions. Secondly, such studies can only be utilised to study single end-points and cannot be utilised to examine cell choices in migration. Thirdly, optical aberrations related to the spherical geometry of the glass capillary sections limit stage-related in situ (post-fixation) analysis of the intracellular structures (cytoskeleton and signalling molecules). Finally and most importantly, the usual observation periods lie between 5-30 minutes for rolling experiments. Longer studies are required to study subsequent crawling steps on endothelial and extracellular matrix ligands. In this regard, studies relating to the effects of chemokines have largely been limited to cellular arrest on adhesion receptor ligands and have not been extended to the study of cell crawling. For example, specific

chemokines have been shown to induce rolling arrest with enhanced binding of lymphocytes to ICAM-1, otherwise known as CD54.

Presently accepted techniques for cell adhesion or binding assays involve the initial coating of a surface of a device with a substrate, typically a protein. Cells are deposited onto the substrate and allowed to settle. Following the settling of the cells, the device is heated to 37°C and is visually analysed using an inverted microscope, or alternatively it is subjected to a stand-alone heating stage and progression of cell binding can be checked at intervals with the inverted microscope. The duration of these assays may be varied depending on the cell line and choice of substratum. Following cell adhesion, free cells may be washed away and a subsequent cell count may be carried out.

Although these methods provide semi-quantitative information regarding a cell type's affinity for a particular substratum, there is no simple method for quantitative characterisation of binding or methods enabling a prolonged study of cell rolling, the ensuing capture by the substratum and subsequent attachment. Furthermore, direct studies of changes in cell morphology, cell growth and biochemical changes cannot be provided easily with these techniques since, determining the kinetics of attachment and resulting morphological changes requires multiple replicated experiments being analysed at different times.

US Patent Specification No. 5998160 (Berens et al) describes a static assay which unfortunately does not have any consideration of cell flow and rolling.

The ability of T-cells circulating in the bloodstream to adhere to the endothelium, switch to a motile phenotype and penetrate through the endothelial layer is recognised as a necessary requirement for the effective in vivo movement or as it is sometimes referred to, trafficking of specific lymphocyte sub-populations. Motility assays are done in combination with attachment assays since following adhesion; cells are expected to switch to the motile phenotype. Motility assays are assessed by estimating the ratio of cells undergoing cytoskeletal rearrangements and the formation of uropods (extension of the trailing tail). One of the major disadvantages of this and the previous adhesion assays is the geometrical design (microscope slides and multiple well chambers), which does not at all resemble the in vivo situation.

The most commonly used cell transmigration assay is a modified "Boyden chamber" assay such as described in US Patent Specification No. 5578492 (Fedun et al). This involves assessing the crossing of a quantity of cells through a microporous membrane under the influence of a chemoattractant, recombinant or cell-derived. Here the diameter of the micropores are less than the diameter of the cells under investigation, such that the cells must deform themselves in order to squeeze through the pores thereby constructing an analogy to the transendothelial migration of cells in physiological circumstances. Once cells are deposited onto the membrane, the chamber can be incubated for intervals over time at a suitable temperature, usually 37°. Following this, the bottom chamber or opposite side of the top chamber may be analysed for cells that have squeezed through the microporous membrane.

US Patent Specification Nos. 4912057 (Guirguis et al), 5284753 (Goodwin et al), 5302515 (Goodwin et al), 5514555 (Springer et al) and 5601997 (Tchao) are typical examples of these assays. The main disadvantage of the assays described in those specifications is that the biological process of transmigration through the micropores is difficult to observe due to the geometrical configuration of the apparatus involved. The lens of the optically inverted microscope must be able to focus through the lower chamber and the microporous membrane. This obviously leads to difficulties due to optical aberrations. In effect, the study of the cells morphology changes while transmigrating across the membrane and their subsequent cytoskeletal changes reverting to their former state is a process which is difficult to monitor and record due to limitations with current techniques. In addition, although it is possible to alter such an experiments parameters following the initiation of the experiment, such as the introduction of a second chemoattractant, recombinant or cell-derived, at some specified time after commencing the experiment, it is not possible to distinguish separate effects from each said chemoattractant.

In addition to cell biology studies, the pharmaceutical industry has major problems in the drug screening process and while high throughput screening (HTS) has been extremely successful in the elimination of the large majority of unsuitable drugs, it has not progressed beyond that and usually, after a successful HTS assay, a pharmaceutical company may still have 7,000 possible drugs requiring assessment.

This requires animal trials and anything that can be done to reduce the amount of animal trials is to be desired. Thus, there is a need for new techniques for drug testing in the pharmaceutical industry. The current proposals are to screen the physiological response of cells to biologically active compounds such as described in US Patent Specification No. 6103479 (Taylor). This again, unfortunately, is still a static test. Since the cells are spatially confined with the drug, there may be a reaction but it may not necessarily take place when the cells are free to flow relative to the drug as in, for example, the microcapillaries of the body. There are other disadvantages such as the transport and subsequent reaction of the drug following its injection into the animal. Probably the most important disadvantage is that it does not in any way test, in a real situation, drug efficacy.

Finally, there are no techniques at the present moment for performing assays to test the interaction of a large number of chosen compounds with living cells while the cells or compounds mimic the in vivo situation of continuous flow.

While in the description herein, the examples all refer to animal cells and indeed mainly human cells, the invention equally applies to plant cells. The term "sample liquid" refers to a suspension of living cells within a suitable carrier liquid which is effectively a culture medium. More than one cell type may be in suspension. Further, the term "reagent liquid" could be any liquid from a drug under assessment, a poison, a cell nutrient, chemoattractant, a liquid containing other cells in suspension or indeed any liquid who's effect the sample liquid requires assessment.

The present invention is directed towards a biochip assembly for performing such assays.

Statements of Invention

The invention provides a biochip assembly comprising a plurality of separate biochips, each comprising:-

an enclosed elongate microchannel having an inlet port adjacent its proximal

end;

an outlet port adjacent its distal end;

a reservoir well for each biochip;

an enclosed main delivery channel feeding a plurality of delivery channels, each having a combined output and input port; and

an enclosed sample holder conduit for connecting a delivery channel for interconnection of the ports and for the reservoir wells.

Ideally, an output reservoir well is associated with each outlet port of each separate biochip and in which an enclosed conduit for the output port of the biochip to its associated output reservoir.

Ideally, each conduit is releasably connected to each of its associated ports and wells.

The conduit may ideally be a length of flexible tubing and normally has an internal cross sectional area substantially greater than that of the microchannel.
In many embodiments, there will be more than two microwells associated with each biochip and indeed, the biochip incorporated therein may be of any of the biochips described above.

Detailed Description of the Invention

The invention will be more clearly understood from the following description thereof, given by way of example only, with reference to the accompanying drawings, in which:-

Fig. 1 is a perspective view of a biochip assembly according to the invention with portions removed,

Fig. 2 is a sectional view through the biochip assembly, and

Figs. 3 to 6 are plan views of biochip assembly in different positions of use.

Referring to Figs. 1 to 6 inclusive, there is illustrated a biochip assembly. The biochip assembly is illustrated and indicated generally by the reference numeral 100 and is manufactured by a clear plastics material, however, certain portions, which can be partially seen in Fig. 1, are illustrated. The biochip assembly comprises a plurality of biochips, indicated generally by the reference numeral 50, each biochip comprises a microchannel 51 having an input port 1 and an output port 3. It will be appreciated that to a certain extent, the term "input port" and "output port" is a misnomer since in one circumstance, a port may operate as an input port and in another circumstance, as an output port. The size of the microchannels 51 can vary in cross section from between 5 μm x 5 μm to 100 μm x 100 μm but will generally exceed 20 μm x 20 μm. The microchannels are generally of the form disclosed in U.S. Patent Application No. 09/750,348.

Individual biochips are not identified by subscript letters as it would only confuse the description. The biochips 50 are manufactured using standard lithographic and hot embossing techniques. A stainless steel substrate is masked with photoresist (SU-8-5 m, as distributed by Chestech). After ultraviolet lithography, the photoresist mask is delivered and the substrate is electrochemically etched to produce a negative master mould in stainless steel. The remaining mask is subsequently removed. Hot embossing is employed to replicate the microfluidic pattern of the microchannels 51 in a variety of thermoplastic materials such as PMMA, polycarbonate, and polystyrene. The liquid inlet and outlet ports, such as the port 1, are glued in position. The biochip is treated in oxygen plasma (0.1 torr, 80% oxygen and +100V for 30 seconds) to ensure a hydrophilic surface and is subsequently sealed with a pressure-sensitive film (PHARMCAL PM-150-c TC-249 V-232C 150 POLY H9, manufactured by Flexcon). This film is a 1.5 mil top-coated clear polyester film, coated with a permanent adhesive containing a photoluminescent additive, backed with a 1.5 mil polyester release liner. The width of the channels may vary from 20-100 μm and a depth from 20-40 μm. The biochip 50 is thus an optically transparent structure. The biochips are generally of the form disclosed in U.S. Patent Application No. 09/750,348.

There is provided a pair of reservoir wells 101 adjacent each inlet port 1 and two output reservoir wells 102 adjacent each outlet port 3. There is further provided a main delivery channel 103 having an inlet port 104 which in turn feeds through further delivery channels 105 each having a combined input/output port 106. An enclosed conduit 107, in this case provided by a length of plastics tubing, interconnects the various ports and/or the wells, as will be described hereinafter. The internal cross-sectional area of the conduit 107 is considerably greater than that of the microchannel 51.

Above the reservoir wells 101 and 102, there is provided conduit supports comprising a bridge plate 108 having holes 109 which is mounted above and spaced-apart from the respective reservoir wells 101 and 102, only one bridge plate 108 is illustrated in Fig. 1.

In operation, for example, when conducting a cell adhesion study, a syringe pump is connected to the biochip assembly 100 through the inlet port 104. For example, different ligands could be provided in each reservoir well 101 and the same cell sample in each of the other of the pair of reservoir wells 101. These could be pipetted in or provided in any known way. Then the conduit 107 is connected between initially the well 101 containing the ligand. The ligand is then drawn into the conduit 107, as illustrated in Fig. 3. Then, the conduit 107 is connected to each of the input ports 1 and the ligand delivered through each biochip 50 to coat the interior of the microchannel 51. After coating, a suitable culture medium solution can be delivered through the main delivery channel 103 and through the combined input/output ports 106 through the conduit 107 and then through the microchips 50 (Fig. 4). Then, the conduits 107 which form sample holders can be disposed of and replaced with new sample holder conduits 107 which would be each dipped into the other of the reservoir wells 101 where the cell suspension will then be aspirated into the conduit 107, as shown in Fig. 5. Then, as shown in Fig. 6, the conduit 107 would be again connected to the input port 1 and a further conduit 107 would be connected to each output port 3 and to one of the output reservoir wells 102, as illustrated in Fig. 6. The assay is then carried out.

It will be appreciated that it would be possible to use a plurality of biochips in series. Thus, for example, rather than one array of biochips, as illustrated, there could be

further arrays of biochips. Further, by having the output reservoir wells 102, it is possible to conduct further post-analysis work on the samples. It will be appreciated therefore that the biochip assembly 100 essentially comprises four sections, namely, the flow splitter section, sample preparation section, analysis and a post-analysis section.

It will be appreciated that the conduits are essentially disposable sample holders. It will also be appreciated that in most cases, biological assays are a multi-stage process and requires consecutive injection of several samples into the one microchannel. Thus, an ability to dispose the sample holder tube or conduit contaminated with one sample and replace it with a new uncontaminated tube, is particularly important. It is also important to avoid the contamination of any of the other parts of the biochip and thus cross contamination.

It will also be appreciated that it is advantageous to be able to collect the samples from the output of the analysis section, that is, where the biochips 50 are situated. In many situations, for example, gene expression of sample cells which did not react with a particular ligand may be required. Similarly, waste of ligand solution can be stored in one of the output reservoir wells. It will also be appreciated that additional reservoir wells may be provided and that further, additional sets of biochips may also be provided.

One of the great advantages of using the biochips in accordance with the present invention is the reduction in reagent or sample consumption. It will also allow reduced analysis times and larger transfer rates due to the diminished distances involved. Additionally, in running several assays in parallel, each process in an assay can be manipulated step by step through computer control enabling great efficiency. Again, this accuracy in combination with higher yields, leads to a reduction in waste. This is not only more economically favourable but also environmentally beneficial where hazardous chemicals are involved.

In addition to chemical production, there are numerous other fields in which the micro devices according to the present invention can make a contribution, such as microbiology, pharmacy, medicine, biotechnology and environmental and materials

science. The present invention is particularly adapted to the field of drug discovery and combinatorial chemistry. Again, there should be considerable cost savings for pharmaceutical companies. One of the great advantages of the present invention is that it mimics in vivo testing. Obviously, with the present invention, there is a constant flow of cells and the drug candidate, together with the micro capillary under observation, produces much more accurate statistical results.

One of the problems with current toxicity tests is that the systems implemented are not always representative of those in vivo providing results which are not characteristic of the in vivo situation. Secondly, there are differences with culturing and maintaining certain cells in vitro. The present invention allows one to simulate in vivo conditions eliminating many of the disadvantages of the present testing and hence immediately decreasing the necessity for animal trials while simultaneously increasing the statistical response as a result of the continuous flow assay according to the present invention.

One of the major problems with all drug testing is that clinical trials involve testing of the new drug in humans and because of the rigorous testing involved in a new drug, the time and cost of bringing a drug to market is enormous. It is for this reason that pharmaceutical companies must be extremely accurate with results obtained through experimental assays before presenting a new drug for clinical trials.

One of the advantages of the present invention is that relatively small volumes of blood can be used for analysis in hospitals which can be extremely advantageous. A particular advantage of the present invention is that the biochips are disposable.

The present invention essentially provides techniques for performing assays that test the interaction of a large number of chosen compounds, for example, candidate drugs or suspected toxic samples with living cells while the cells and/or the compounds mimic the in vivo situation of continuous flow. The assays according to the present invention imitate as far as possible the natural situation, while additionally overcoming the disadvantages of other techniques resulting in a fast and accurate process.

It will be appreciated that since the biochips are fabricated from a plastics material, it is considerably less expensive than, for example, silicone micro-machining which is often

used at present, for such microchips.

One of the great advantages of plastics material is that it enables real-time monitoring with relative ease, by use of a inverted microscope.

The size of the microchannels is also significant. Dimensions below the order of 1 mm have long be avoided due to the many difficulties that occurred when scaling down. Such difficulties involve the control of flow within these microchannels.

While in the present invention, many tests have been tried and described, it will be appreciated that many other assays and tests can be carried out in accordance with the present invention. Indeed, some of the tests according to the present invention are not so much tests, as indeed filtering operations.

In the specification the terms "comprise, comprises, comprised and comprising" or any variation thereof and the terms "include, includes, included and including" or any variation thereof are considered to be totally interchangeable and they should all be afforded the widest possible interpretation.

The invention is not limited to the embodiments hereinbefore described but may be varied in both construction and detail.

CLAIMS

1. A biochip assembly (100) comprising a plurality of separate biochips (50), each comprising:-

   an enclosed elongate microchannel (51) having an inlet port (1) adjacent its proximal end;

   an outlet port (3) adjacent its distal end;

   a reservoir well (101) for each biochip;

   an enclosed main delivery channel (103) feeding a plurality of delivery channels (105), each having a combined output and input port (106); and

   an enclosed sample holder conduit (107) for connecting a delivery channel (105) for interconnection of the ports (106, 1, 3) and for the reservoir wells (101, 102).

2. A biochip assembly (100) as claimed in claim 11 in which an output reservoir well (102) is associated with each outlet port (3) of each separate biochip (50) and in which an enclosed conduit (107) for the output port (30) of the biochip (50) to its associated output reservoir (101) is provided.

3. A biochip assembly (100) as claimed in claim 11 or 2 in which each conduit (107) is releasably connected to each of its associated ports (106, 1, 3) and wells (101, 102).

4. A biochip assembly (100) as claimed in any of claims 1 to 3 in which the conduit (107) is a length of flexible tubing.

5. A biochip assembly (100) as claimed in any of claims 1 to 4 in which the conduit (107) has an internal cross-sectional area substantially greater than that of the

microchannel.

6. A biochip assembly (100) as claimed in any of claims 1 to 5, in which there is more than two microwells (101, 102) associated with each biochip.

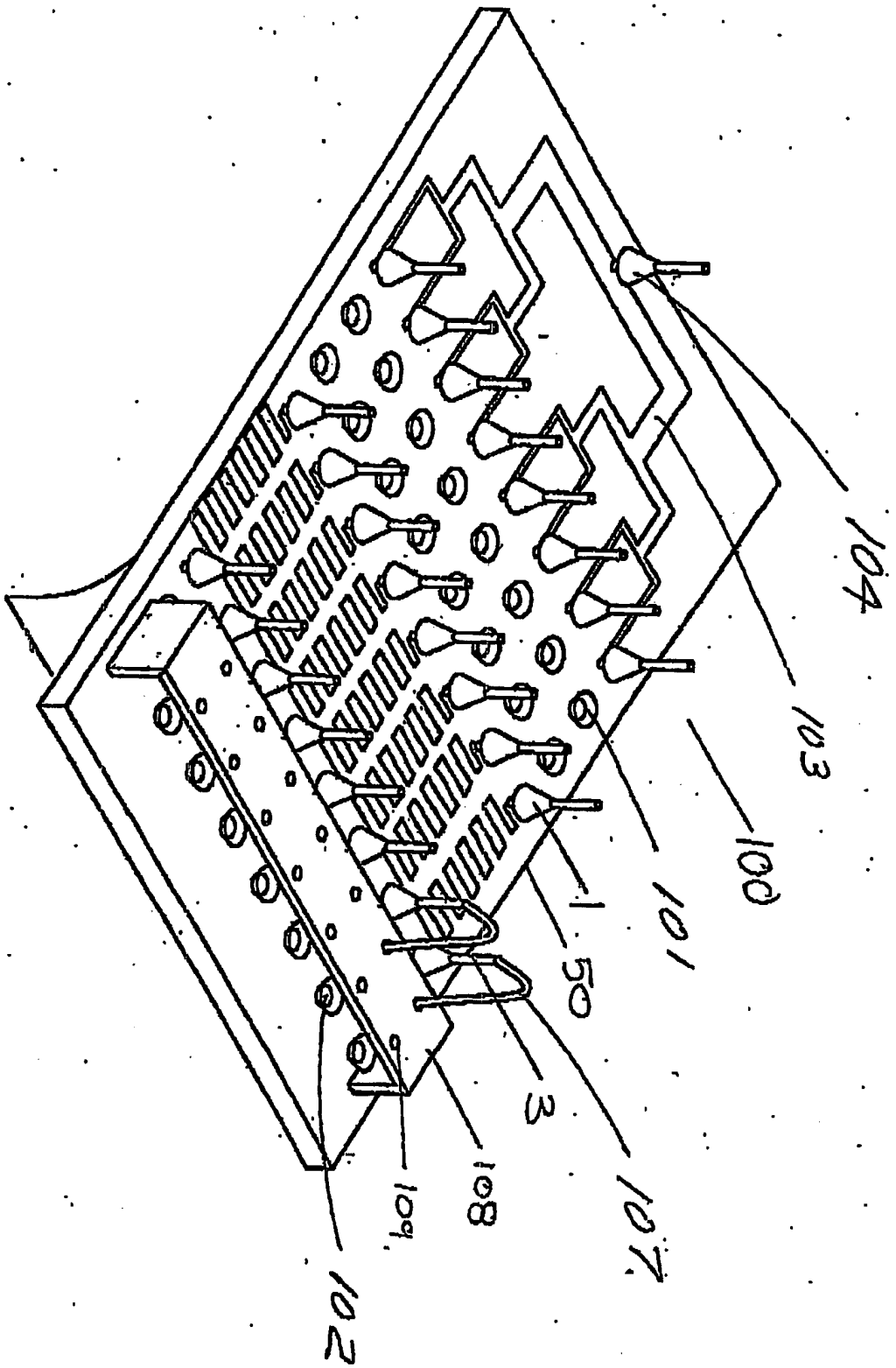

Fig. 1

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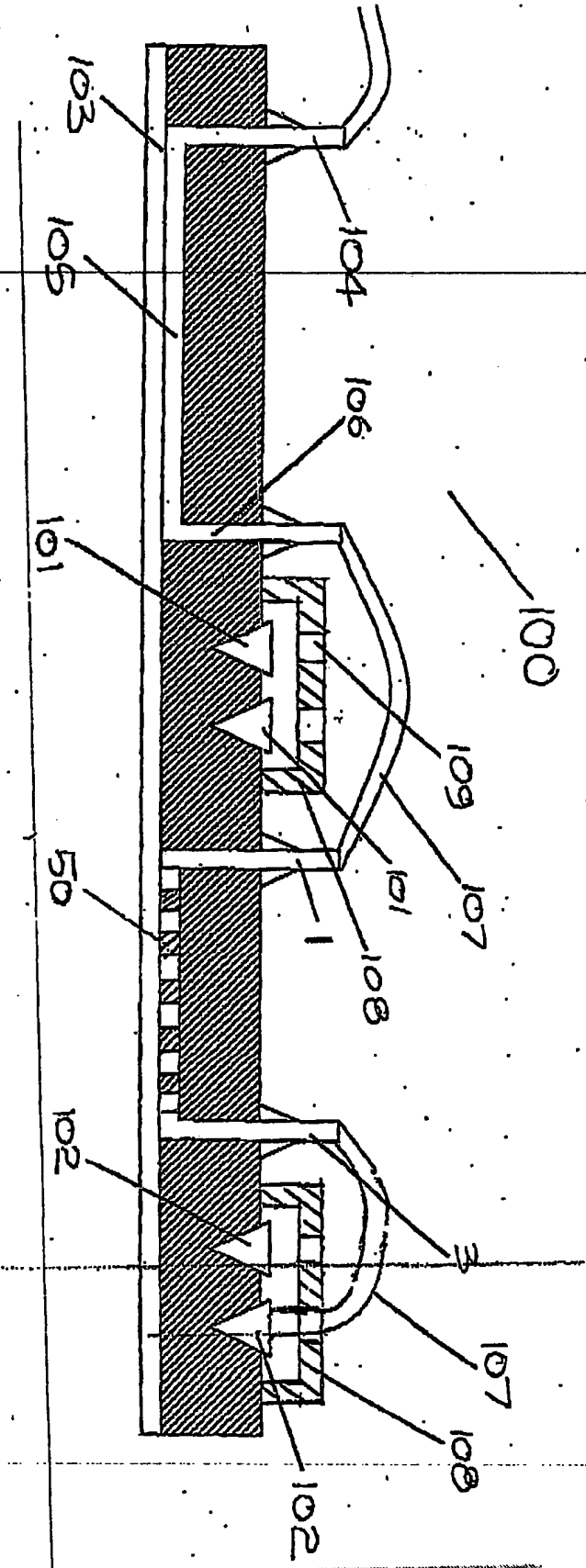

Fig 2

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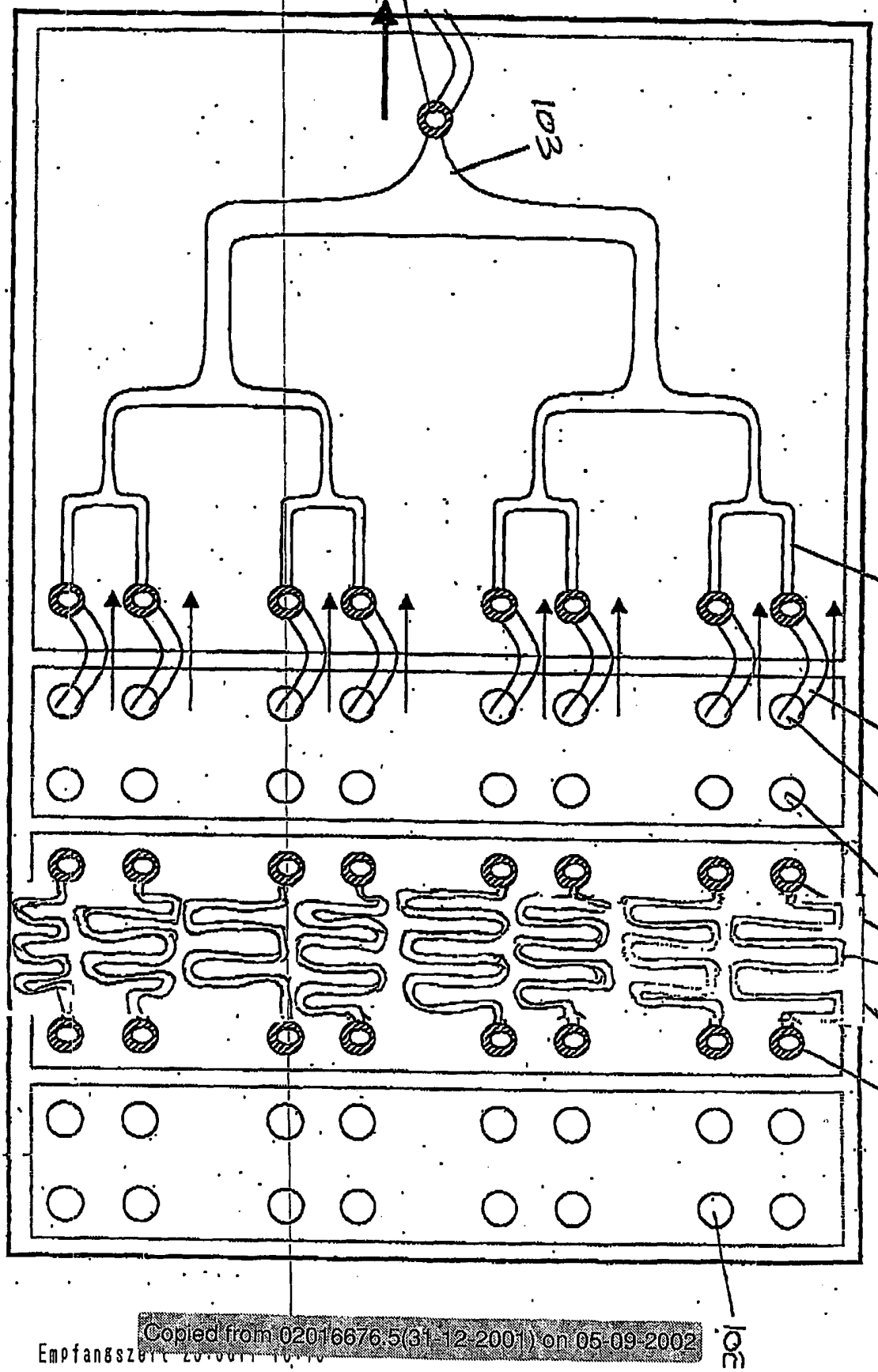

Fig 3

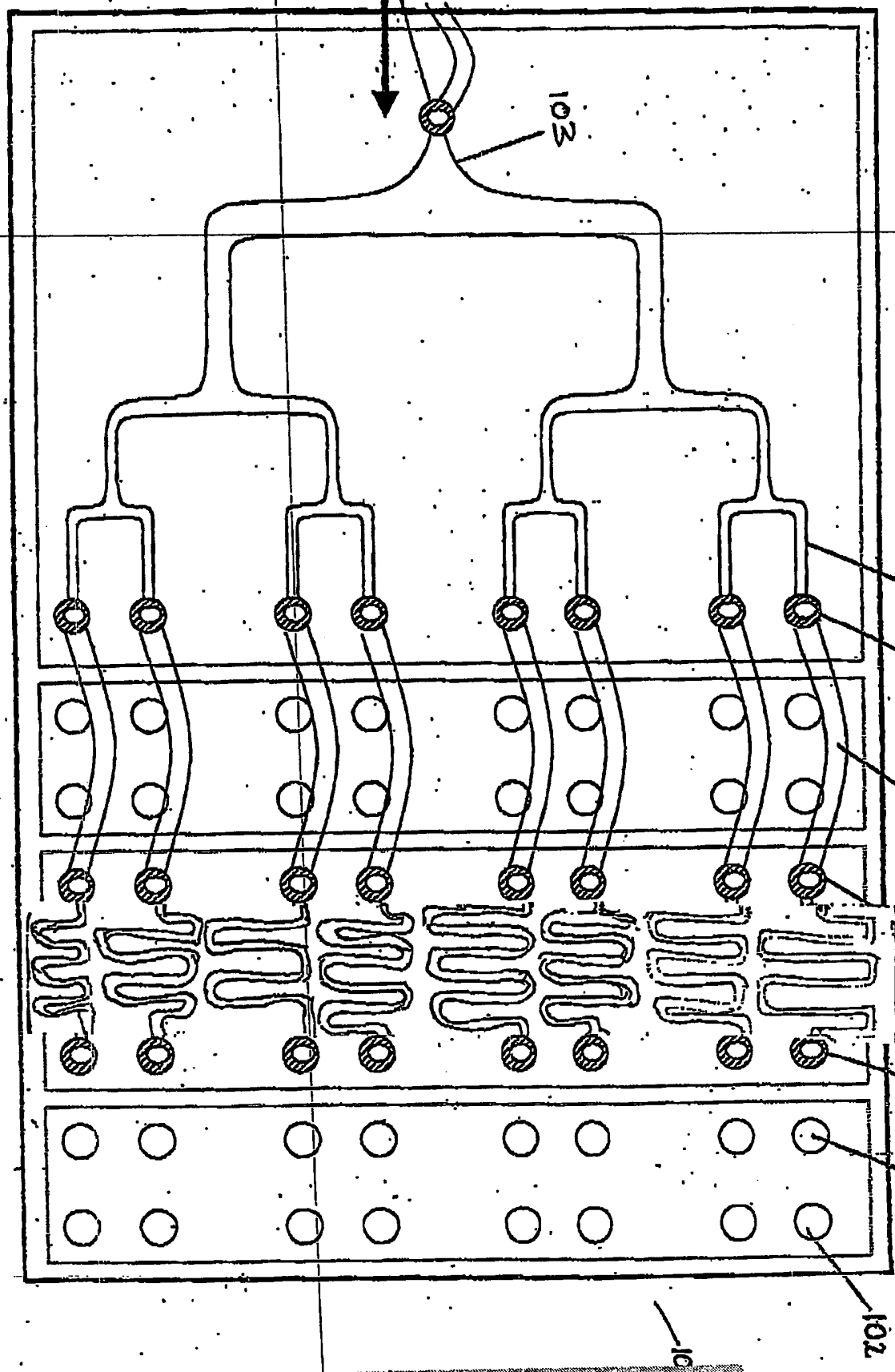

Fig 4.

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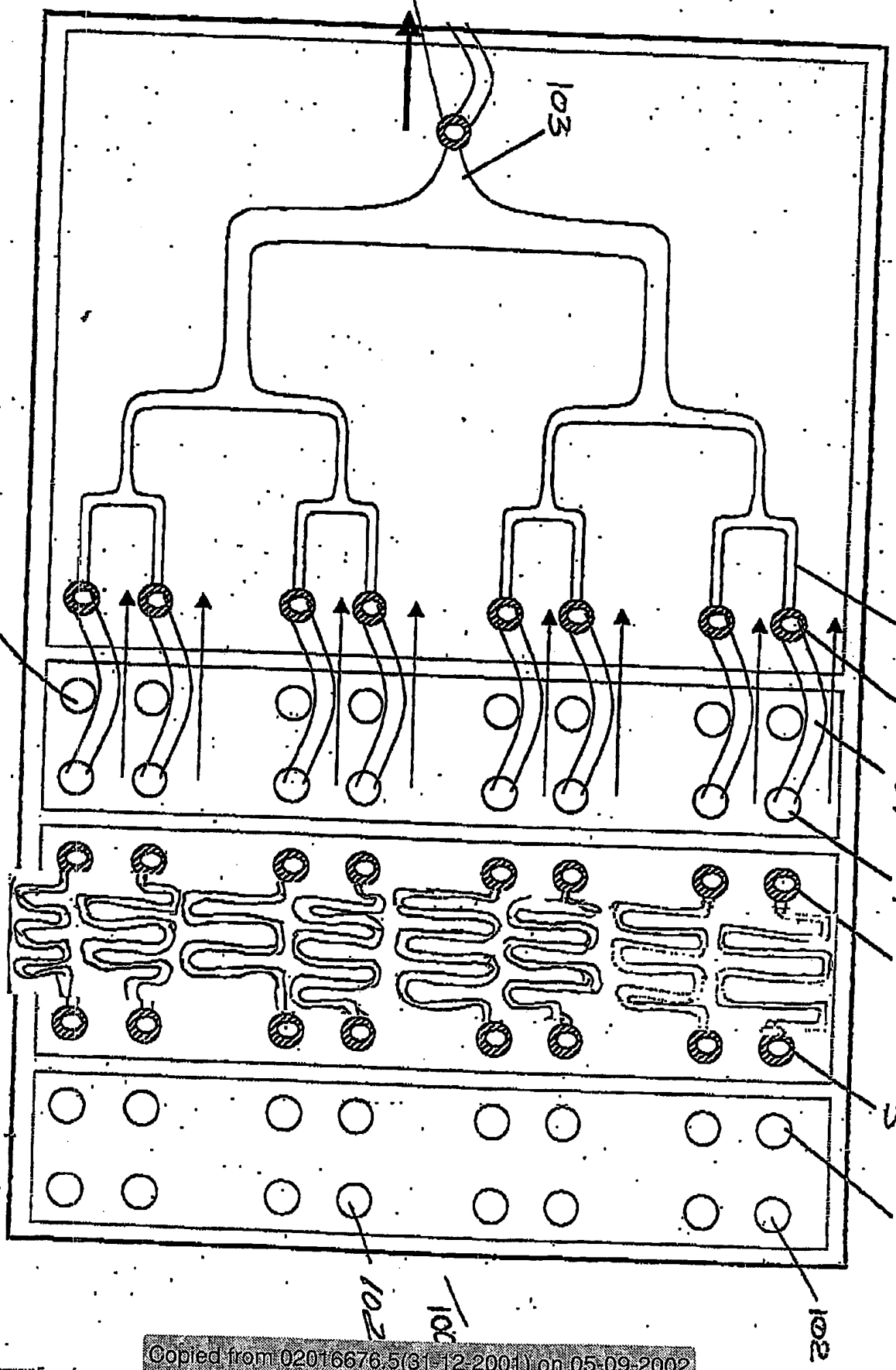

Fig 5

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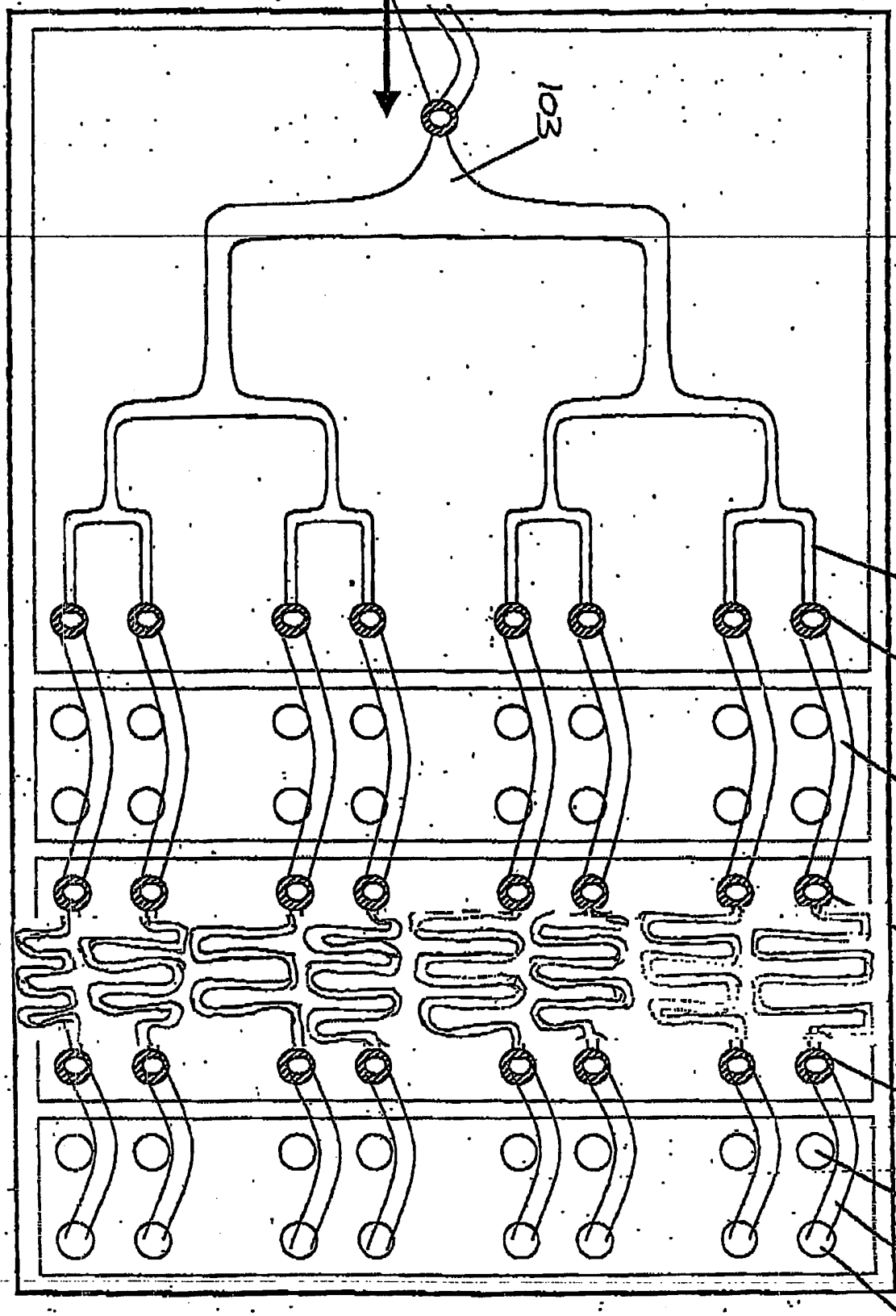

Fig 6

ABSTRACT

"Biochip Assembly"

(Fig. 1)

A biochip assembly 100 comprising a plurality of separate biochips for sue in biological assays is disclosed. Each biochip on the assembly comprises a microchannel 51 having an inlet port 1, an outlet port 3, a reservoir well 101, an enclosed main delivery channel 103 and an enclosed sample holder conduit 107. In operation a ligand may be provided in each reservoir well 101 to coat the interior of the microchannel. A syringe pump is connected to the biochip assembly 100 through the inlet port 104 to deliver a suitable culture medium solution through the main delivery channel 103, in order to perform an assay.

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